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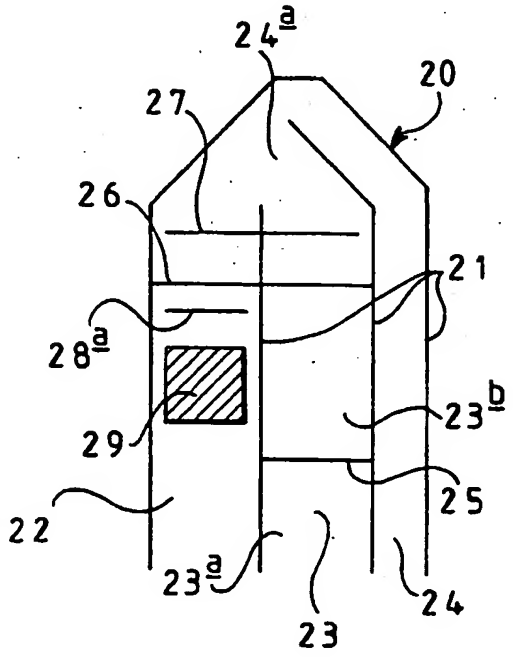
(54) Title: FLUID-FLOW CONTROL DEVICE



(57) Abstract: The present invention resides in one aspect in an assay device for an analyte comprising a flow path (22, 23, 24) for a liquid defined by interstices in a porous medium (20), at least one liquid flow barrier (26) which is impregnated into and immobilised on the porous medium (20) at a location in the flow path (22, 23, 24), at least one barrier release means (27) which is impregnated into the porous medium (20), and an analyte capture region (28a) in the flow path (22, 23, 24). The barrier release means (27) is soluble in the liquid, and, in use, is moveable by liquid flowing in the flow path (22, 23, 24) into contact with the flow barrier (26) so as to permit liquid flow through said location. Preferably, an analyte visualising agent is provided at a zone (29) in the flow path (22, 23, 24), said visualising agent being capable of interacting with the analyte to indicate the presence of the analyte.

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FLUID-FLOW CONTROL DEVICE

The present invention relates in a first aspect to a fluid-flow control device.

There are a large number of assay devices for determining chemical or biochemical analytes in a sample. One common type of device comprises a strip of porous material impregnated in at least one discrete region with a reagent sensitive to the analyte of interest. The reagent is usually chosen so that the presence of the analyte is readily observable (eg. appearance or change in colour or fluorescence). A sample is applied to one region of the strip and liquid (eg. water) is applied to the same or another region. As an alternative, the sample may be the liquid or contained in the liquid. The liquid is drawn by capillary action along the strip and carries the sample through the region(s) containing the reagent(s), until saturation of the strip occurs when liquid reaches the far end of the strip. Any analyte of interest present in the sample will contact the reagent(s) as the liquid passes along the strip. Such devices may include built-in procedural controls and/or end of test indicators which are well known in the art.

One disadvantage of such devices is that the contact period between sample and reagent is dependent upon the rate of flow of the sample across the region(s) containing the reagent(s). For certain analyte/reagent pairs, this period may not be long enough for sufficient reaction to occur, i.e. such a device lacks the necessary sensitivity to measure the presence of certain analytes.

US 5874216 discloses a device having a chromatographic strip and a separate sample preparation region on opposable components which can be brought into opposition. The sample preparation region may incorporate a reagent with which the analyte of interest binds and after an appropriate reaction time the opposable components are brought together so that the sample contacts the chromatographic strip and flows into a detection zone.

A successful assay may be dependent upon the user allowing the correct time to elapse between sample preparation and application of sample to the chromatographic strip.

US 4727019 discloses a device which partly overcomes the above disadvantage. The device comprises a porous membrane in contact with an absorbent member. The porous membrane is impregnated with a receptor for a ligand of interest. Sample containing the ligand of interest is applied to the upper side of the membrane and is drawn through the membrane into the absorbent member by capillary action. A labelled receptor which also binds the ligand of interest is then applied to the porous membrane to react with the receptor-ligand complex so that the receptor-ligand complex may be observed. Sensitivity of the reaction may be controlled by applying a large amount of sample to the membrane and/or allowing a longer time period between applying the sample and the labelled receptor.

However, disadvantages are (i) the use of such a device may require large amounts of sample, (ii) the sample and labelled ligand are applied to the

membrane manually and (iii) the period between application of the sample and ligand must be measured.

None of the prior art devices are able to control the assay by controlling the flow of the liquid along the porous medium.

Thus, an object of the present invention is to provide a fluid-flow control device capable of use as an assay device eg. for determining chemical or biochemical analytes present in a sample. Another object is to provide a device which obviates or mitigates one or more disadvantages of the prior art assay devices.

According to a first aspect of the present invention, there is provided a fluid-flow control device comprising:-

(i) a fluid flow path,

(ii) a fluid flow barrier at a location in the flow path, and

(iii) barrier release means in the flow path which, in use, is moveable by fluid flowing in the flow path into contact with the flow barrier so as to permit fluid flow through said location.

It will be understood that there is no flow through said location until the barrier is contacted in use by the barrier release means.

Preferably, said fluid is a liquid and more preferably an aqueous liquid.

Preferably, the fluid flow barrier is a non-wetting region in the flow path and the release means is a wetting agent capable of wetting said non-wetting region, so as to enable flow of liquid through said region.

Preferably, the fluid flow barrier is a hydrophobic material and the release means is a surfactant. Examples of suitable hydrophobic materials include waxes (e.g. paraffin wax), resins (e.g. acrylic resin based varnish, 100% acrylic polymer varnish, coumarone-indene resin) and commercially available synthetic polymers, inks and paints. Examples of suitable surfactants include octyl-β-D-glucopyranoside (ODG), dioctyl sulfosuccinate sodium salt (DOSS), polyoxyethylene (23) dodecyl ether (BRIJ 35) and polyoxyethylene (20) sorbitan monolaurate (TWEEN 20).

Preferably, the flow path is defined by interstices (i.e. capillaries and/or pores) of a porous medium. More preferably said porous medium is a nitrocellulose-based material.

Preferably, the fluid flow barrier is impregnated into and immobilised on the porous medium. Preferably, the barrier release means is impregnated into the porous medium and is soluble in the flowing fluid.

Preferably, the device is constructed so that, in use, there is at least one region in the flow path where the direction of fluid flow is different, after fluid flow through said location is initiated compared with the direction of fluid flow in that region before fluid flow through said location is initiated. More preferably the fluid flow direction is reversed.

The present invention also resides in an assay device for an analyte comprising

(i) a flow path for a liquid defined by interstices in a porous medium,

(ii) at least one liquid flow barrier which is impregnated into and immobilised on the porous medium at a location in the flow path,

(iii) at least one barrier release means which is impregnated into the porous medium, which is soluble in the liquid, and which, in use, is moveable by liquid flowing in the flow path into contact with the flow barrier so as to permit liquid flow through said location, and

(iv) an analyte capture region in the flow path.

Preferably, an analyte visualising agent is provided at a zone in the flow path, said visualising agent being capable of interacting with the analyte to indicate the presence of the analyte. Alternatively, the device may be provided with a visualising agent application zone adapted to receive a visualising agent.

As used herein, the expression "visualising agent" encompasses any agent which interacts with the analyte directly or indirectly via an intermediary analyte binding reagent (eg. enzyme labelled conjugate) and which enables the visual or instrumental detection of analyte qualitatively, semi-quantitatively or quantitatively eg. by colour, fluorescence, luminescence or radioactivity. It will be understood that the visualising agent may be inherently detectable, or detectable once interaction with the analyte or intermediary analyte binding reagent has occurred.

In a first embodiment, the visualising agent is immobilised in the capture region and also serves to immobilise the analyte in the capture region.

In a second embodiment, the analyte capture region includes an immobilised analyte binding substance which serves to immobilise analyte in the capture region and the visualising agent is upstream of the capture region so that in use, it flows through the capture region.

The device may include a sample application zone in the flow path which may, if present, correspond to the analyte capture region or the visualising agent application zone. The sample application zone may be surrounded by an additional flow barrier and an associated barrier release means. Alternatively, the sample to be analysed may be included in the liquid which flows, in use, along the flow path.

In a third embodiment, at least two barriers and at least two barrier release means are provided and arranged so that, in use, some liquid flows over the analyte capture region at least twice.

In a fourth embodiment, a flow path geometry is selected which causes, in use, a predetermined difference in fluid flow within the analyte capture region so that distribution of analyte within the analyte capture region is non-uniform in a predictable manner, whereby the amount of analyte can be determined in at least a semi-quantitative manner.

In those embodiments where more than one barrier is present, the barriers may have different constitutions to each other. Similarly where more than

one barrier release means is present, the barrier release means may have different constitutions to each other.

Embodiments of the invention will now be described by way of Example only, with reference to the accompanying drawings, in which:-

Figure 1 show a flow control device in accordance with the present invention used to test various barriers and surfactants,
Figures 2a, 2b and 2c show assay devices in accordance with the present invention for incubating a sample and reagent at a capture site (Figure 2a), or for incubating a sample and reagent before flowing over a capture site (Figure 2b), or for improving the uniformity of switching barriers in devices of the type shown in Figures 2a and 2b.
Figure 3 shows an assay device in accordance with the present invention for causing repeated sample flow across a capture site,
Figure 4 shows an assay device in accordance with the present invention for first delivering a large sample volume through a capture site, followed by analyte labelling,
Figure 5 shows an assay device in accordance with the present invention for isolating a sample region from its surroundings,
Figure 6a shows a membrane strip for use in an assay device in accordance with the present invention where a relatively large volume of sample moves vertically through the thickness of the strip and horizontally along the strip,
Figure 6b is a sectional view of the assay device incorporating the strip of Figure 6a,

Figure 7a shows an assay device in accordance with the present invention where a relatively large volume of sample moves vertically through the thickness of a membrane and is directed by a coacting wicking member to cause horizontal flow along said membrane,

Figure 7b is a cross section through the device at Figure 7a,

Figures 7c to 7e show the sequence of assembly of the device of Figures 7a and 7b, and

Figure 8 shows a semi-quantitative assay device in accordance with the present invention, in which the line length of a visual signal indicates the amount of analyte present in a sample.

In the following Examples, the use of "up", down", "left" and "right" and other reference directions relates to the orientations shown in the drawings.

EXAMPLE 1 Porous media, barrier and surfactant interaction

Referring to Figure 1 a polyester supported nitrocellulose membrane sheet of 3 µm or 8 µm nominal pore size (Whatman International Ltd, Maidstone England) was cut into a 10 mm wide by 60 mm long strip 11 with a barrier 12, 20 mm from one end 13. The barrier 12 was applied to the sheet using a Type MP5200 flat bed plotter (Graphtec Corporation, Yokohama, Japan) prior to cutting. A short perpendicular line 16 was drawn manually at each end of the barrier 12 along the cut edges of the strip 11 using a Lumocolor pen. The strip 11 was dried for 10 minutes at 35° C in an air circulating oven before use. Several such strips 11 were prepared from each sheet and strips were stored in sealed containers prior to use.

In use, the end 13 of the strip was dipped vertically into about a 3 mm depth of liquid (water or surfactant-containing water, the surfactant serving as a barrier release means), in a vessel 14. Liquid wicked up the membrane to the barrier 12 and the time taken for the liquid to penetrate ("switch") the barrier 12 was noted. The pen lines 16 prevented liquid circumventing the barrier and wicking up the cut edges of the strip 11. To see the passage of liquid more clearly a blue line 15 was manually drawn just below the barrier using a water soluble 0.6 mm "Note Writer" pen (Berol, Banford, Norfolk,UK).

The flow characteristics of different combinations of membrane, barrier and surfactant were investigated. Tables 1 to 11 show for each combination (i) the maximum permissible concentration (%) of surfactant in the liquid (water) to delay barrier switching by at least ten minutes (max conc. %), (ii) the concentration (%) of surfactant required to allow rapid switching of the barrier (switching conc. %), and (iii) the transition time (switching time) across the barrier for liquid at the same concentration as (ii), defined as the time in seconds from liquid touching the underside of the barrier to when it starts to appear on the opposite side of the barrier (in the absence of barrier the transition time is <5s).

Table 1 - Yellow Pen Barrier[1] (8 µm membrane)

	ODG[2]	DOSS[3]	BRIJ 35[4]	TWEEN 20[5]
max conc. %	0.03	0.015	0.5	0.5
switching conc. %	0.125	0.06	2.5	5
Transition time (s)	16	14	60	43

[1]Paint Marker Type 751 (Edding AG, Ahrensburg, Germany)
[2]ODG (Octyl-β-D-glucopyranoside) (Fluka, Buchs, Switzerland).
[3]DOSS (Dioctyl sulfosuccinate, sodium salt) (Sigma-Aldrich Co Ltd, Poole, UK).
[4]BRIJ 35 (Polyoxyethylene (23) dodecyl ether) (ICN Biolchemicals, Cleveland, USA).
[5]TWEEN 20 (Polyoxyethylene(20)sorbitan monolaurate) (Sigma-Aldrich Co Ltd.)

Table 2 - Green Pen Barrier[1] (8 µm membrane)

	ODG	DOSS	BRIJ 35	TWEEN 20
max conc. %	0.03	0.015	0.5	0.5
switching conc. %	0.25	0.06	2.5	5
Transition time (s)	16	14	60[2]	43

[1]Paint Marker Type 780 (Edding AG) fitted with a tip from pen type 751.
[2]5% BRIJ 35 has the same transition time but flows more slowly thereafter

Table 3 - White Pen Barrier[1] (8 µm membrane)

	ODG	DOSS
max conc. %	0.125	0.03
switching conc. %	1.25	0.125
Transition time (s)	11	26

[1]Paint Marker Type 751
(5% TWEEN and 5% BRIJ showed slow uneven switching.)

Table 4 - Silver Pen Barrier[1] (8 μm membrane)

	ODG
max conc. %	0.125
switching conc. %	1.25
Transition time (seconds)	10

[1]Paint Marker Type 751
(5% TWEEN 20, 5% BRIJ and 1% DOSS did not penetrate the silver barrier.)

Table 5 - Yellow Pen Barrier (3 μm membrane)

	ODG	DOSS	BRIJ 35
max conc. %	0.06	0.03	1.25
switching conc. %	2.5	1.0	5
Transition time (s)	9	45	45

(2.5% TWEEN 20 gave slow and uneven switching)

Table 6 - Green Pen Barrier (3 μm membrane)

	ODG	DOSS	BRIJ 35
max conc. %	0.06	Not Done	1.25
switching conc. %	2.5	1.0	5
Transition time (s)	11	60	45

(2.5% TWEEN 20 showed slow and uneven switching)

Table 7- White Pen Barrier (3 μm membrane)

	ODG
max conc. %	0.125
switching conc. %	2.5
Transition time (s)	12

(5% TWEEN, 5% BRIJ and 1% DOSS showed slow & uneven switching)

Tables 1 to 7: Plotting speed, 1cm/s.

Table 8 – Lumocolor[1] diluted 1 in 4 with 1-propanol (8 μm membrane)

	ODG	ODG
max conc. %	0.125	0.125
switching conc. %	2.5	1.25
Transition time (s)	5	60

[1]Staedtler, Nuremberg, Germany

Table 9 - Lumocolor diluted 1 in 2 with 1-propanol (8 μm membrane)

	ODG	ODG
max conc. %	0.125	0.125
switching conc. %	2.5	1.25
Transition time (s)	5	180

Tables 8 and 9: Plotting speed, 5 cm/s, pen size, 0.5 mm.

Table 10 - Coumarone resin[1] (8 μm membrane)

Barrier line width (mm)	% ODG 1.25	0.062	% TWEEN 20 0.5
1.4[2]	5	>600	9
3.8[3]	7	>600	24
4.8[4]	13	>600	111
	Transition time (s)		

[1]Prepared by dissolving: 1 g poly coumarone-co-indene resin, Mn 1090 (Sigma-Aldrich) with 4 g dioxane to give a 20% wt/wt stock solution. The working solution was 0.5 ml of stock solution to which was added 3x0.5 ml heptane mixing between each addition (resultant solution: 5.27% resin wt/vol solvents). Barriers were dried at 35°C prior to testing with surfactants.

[2]single line plotted with 0.5 mm pen at 5 cm/s

[3]single line plotted with 0.5 mm pen at 1 cm/s

[4]three lines, 1 mm apart, plotted with 0.5 mm pen at 1 cm/s

Table 11- Wax Barrier (8 µm membrane) - Transition time

% ODG	% Wax[1] in white spirit			
	1.25	2.5	5	10
1.25	~125	~55	~92	~71
2.5	17	~24	11	~12
5	19	20	8	29
10	33	21	19	~76
	Transition time (s)[2]			

[1]Paraffin Wax mp 51-53°C (BDH, Poole UK) solubilised in white spirit (BDH) by heating gently on a hot plate set at 50°C.
[2]Using 8 µm membrane, all wax barriers, from 1.25 to 10% concentration prevented the flow of deionised water for at least 10 minutes (duration of test). Wax barriers below 0.75% wax allow passage of water. 10% TWEEN 20, 10% BRIJ 35, and 1% DOSS did not penetrate the 1.25% or 10% wax.
"~" = Mean time because the transition was not even along the wax line.

Table 12 shows the distance wicked for the wax barrier/ODG surfactant combinations shown in Table 11.

Table 12- Wax Barrier (8 µm membrane)- Distance wicked

% ODG	% Wax			
	1.25	2.5	5	10
1.25	28	36	35	31
2.5	38	49	51[2]	37
5	33	43	47	30
10	26	35	38	26
	Wicking distance (mm) 5 minutes after immersion[1]			

[1]Without a wax barrier the wicking distance of water was 71 mm
[2]5% wax, 2.5% ODG is the optimum combination in respect of rapid switching and distance wicked

Tables 11 and 12: Plotting speed 10 cm/s, pen size 0.5 mm.

Cryla Soluble Gloss Varnish (Daler Rowney, Berkshire, UK) (an acrylic resin based varnish): Using the 8 µm membrane a barrier of 0.1% varnish in white spirit (plotted at 10cm/s using a 0.7 mm pen) held back water for at least 10 minutes. A line of 2.5% ODG was applied manually (0.7 pen) about 5 mm below the Cryla line and water applied to the end of the strip. The barrier switched in about 12 seconds but flow through the barrier was uneven compared with other barrier materials.

Liquitex High Gloss Varnish (Brinney & Smith Inc., Easton, USA) (100% acrylic polymer varnish): Using the 8 µm membrane a barrier of 80% varnish in water (plotted at 10 cm/s using a 0.7 mm pen) held back water for at least 10 minutes. A line of 2.5% ODG was plotted about 5 mm below the varnish (5 cm/s, 0.7 mm pen) and water applied to the end of the strip. The barrier switched immediately. 70% varnish is marginal as a water barrier because of slight water absorption. This is seen as a bloom on the surface of the varnish after it was applied to an impervious surface, dried, dipped into water for 30 minutes, dried and examined. However, switching was much faster than any of the other barriers and, using an appropriate application method, could be a useful barrier.

Wax and 3µm membrane Wax is difficult to use as a barrier on the 3 µm membrane because wax at a concentration of 0.3% allows water to pass through, but at a concentration of 0.6%, water will not pass through the barrier even at 5% ODG. Thus, the useful working range is very small and the wax content of the barrier critical.

Discussion:

It will be seen that some combinations of porous media, barrier and surfactant result in faster switching of the barrier than others, e.g. 5s for Lumocolor barrier and 2.5% ODG compared with 17s for 1.25% wax barrier and ODG at the same concentration on the same membrane (Table 8 cf. Table 11). For some combinations, switching occurs at a relatively low concentration of surfactant, e.g. 0.06% DOSS (see Table 1) and some surfactants are ineffective in breaking through some barriers, e.g. TWEEN, BRIJ and DOSS through wax barrier (note 2 to Table 11). From the above data, it is clear that a suitable combination can be chosen for the particular analytical system of interest. For example, if it were necessary to have a first surfactant present as part of the analytical reaction(s) but desirable that this surfactant should not cause switching of the barrier, an appropriate choice may be wax for the barrier and TWEEN 20, BRIJ or DOSS for the first surfactant. switching of the barrier could then be controlled by a second surfactant such as ODG.

The flow control devices of Example 1 do not necessarily reflect the performance of practical assay devices, but are intended to illustrate the difference in behaviour of various barrier/surfactant/membrane combinations. In a preferred assay device the surfactant is dried into an area on the membrane during manufacture. In use, the concentration of surfactant at the barrier is dependent upon the concentration of surfactant applied, its volume, geometry, and the geometry and size of the surrounding structure. Additionally, the switching time is not merely dependent upon the nature of the barrier, surfactant and membrane materials, it is also affected by the degree of saturation in the area of

surfactant adjacent to the barrier (saturation is the amount of liquid present per unit area of the membrane). This in turn is dependent upon the geometry and size of the device. This saturation effect can be put to important use to form a feedback-like mechanism so that, for example, a barrier may open only when the device is substantially saturated.

The following Examples show how the principles described above can be incorporated into practical assay devices for determining, for example, chemical or biochemical analytes present in samples such as water, urine and blood. It will be understood that the specific reagents used will depend upon the specific analyte(s) of interest.

EXAMPLES 2a and 2b

Referring to Figure 2a, an assay device comprises a generally rectangular strip of polyester backed 8 μm nitrocellulose membrane 20. Permanent impervious lines 21 formed by the application of neat Lumocolor ink using the pen plotter divide the membrane into first, second and third channels 22,23,24 which extend from the lower end of the device toward its upper end. The second channel 23 is further divided into a lower portion 23a and an upper portion 23b by a horizontal impervious line 25. At their upper end, the first and second channels 22,23 merge into an enlarged common region 24a which separates the upper end of the third channel 24 from the upper ends of the first and second channels 22,23. A switchable barrier 26 (silver paint from pen type 8700 (Edding AG, plotted using a 0.7 mm pen at 1cm/s, and dried at 22° C for 2 minutes) extends across the full width of the first channel 22 and the upper portion 23b of the second channel 23. Spaced above the switchable barrier 26, a line of

surfactant 27 (2.5% ODG plotted using a 0.7 pen at 3 cm/s, and dried at 35° C for 20 minutes) extends substantially across the width of the first and second channels 22,23. Spaced below the barrier 26, a capture line 28a comprising an antibody to the analyte of interest immobilised onto the membrane 20, extends substantially across the width of the first channel 22. Spaced below the capture line 28a, also in the first channel 22, is a region 29 containing gold conjugate for labelling the analyte of interest if present in a sample. A solution of methylcellulose is applied to the membrane prior to the addition of the gold conjugate to prevent unspecific binding to the membrane.

In this embodiment, the analyte is one which can form an immunoassay complex such as a protein, eg. for diagnosis of human chorion gonadotropin (hCG), leutinising hormone (hLH) or C-reactive protein (CRP).

In use, the lower end of the device is placed into liquid so that liquid flow ("wicking") is initiated in the first, second and third channels 22,23,24. The liquid may serve as a diluent for a sample to be analysed or it may be the undiluted sample (e.g. urine). In the first channel 22, liquid wicks up to the gold conjugate containing region 29 where the analyte of interest reacts (if present) to form a gold-analyte complex. Liquid flow continues and the gold-analyte complex (as well as any unreacted gold conjugate) is moved by the liquid flow over the capture line 28a until flow is stopped by the barrier 26. The gold-analyte complex is immobilised at the capture line 28a by binding to the antibodies at the capture line 28a. Liquid also flows in the lower portion 23a of the second channel 23, but is prevented

from entering the upper portion 23b of the second channel 23 by the impervious line 25 therebetween.

While liquid is stationary in the first channel 22 at the barrier 26 and in the second channel 23 at the line 25, liquid continues to flow into the third channel 24 and down into the common region 24a. The liquid front solubilises the surfactant line 27 and when the liquid reaches the upper side of the switchable barrier 26, the surfactant switches the barrier 26 i.e. converts the capillary structure of barrier 26 from non-wetting to wetting enabling liquid to flow therethrough.

The geometry of the device is such that downward flow from the common region 24a into the first and second channels 22,23 is substantially symmetrical, so that the barrier 26 is switched substantially simultaneously in the first and second channels 22,23. Liquid is rapidly drawn into the dry upper portion 23b of the second channel 23 from the common region 24a which in turn draws liquid from the first channel 22 through the common region 24a into the upper portion 23b of the second channel 23. Hence, any unbound material in channel 22 flows quickly into region 24a, further flow acting to wash the capture line 28a. A small volume of liquid also flows from the third channel 24, through region 24a, into the upper portion 23b of the second channel 23. If the analyte of interest is present in a predetermined amount it will be seen as a coloured line at the capture line 28a. Liquid continues to flow in the first and third channels 22 and 24 until the upper portion 23b of the second channel 23 is saturated or the device is removed from the liquid.

Referring to Figure 2b, a modified assay device is shown which is identical to that shown in Figure 2a except that the capture line 28b is disposed in the first channel 22 between the barrier 26 and the surfactant line 27. The modified device is particularly useful when the reaction time between analyte and visualising agent (in this embodiment gold-conjugate) is critical, prior to their flow over the capture line 28b.

In use, about 10 µl of sample is applied onto the gold conjugate containing region 29. The device is placed in liquid and liquid flow is as described with reference to the embodiment of Figure 2a. However, since the capture line 28b is above the barrier 26 in this embodiment, the sample and gold conjugate incubate prior to flowing over the capture line 28b. As before, after a predetermined time the barrier 26 switches causing the gold-analyte complex to flow over the capture line 28b. In this embodiment, it is not necessary for methyl cellulose to be applied to the second or third channels 23,24.

Referring to Figure 2c, an assay device closely resembling that of Figure 2b is shown, the only structural difference being that an additional impervious line 21a is provided partially across the upper end of the first and second channels 22,23 where they merge into the common enlarged region 24a. The additional impervious line 21a is perpendicular to and touches the end of the impervious line 21 defining the first and second channels 22,23 (i.e. said impervious lines 21,21a form a "T" shape). In this particular embodiment the switchable barrier 26 is coumarone resin (plotted using a 0.7 mm pen at 4cm/s) and the surfactant is 2.5% ODG (plotted using a 0.7 mm pen at 5 cm/s). In addition, the surface of the nitrocellulose is covered

with a protective transparent film (0.127 mm thick type 8192 film coated on one side with acrylic adhesive AS-110, Adhesives Research Ireland Ltd., Limerick, Ireland).

In use, it was found necessary to prewash and dry the membrane prior to device fabrication to prevent premature opening of the barrier when the combination of coumarone resin and ODG are used. This is thought to be due to unspecified surfactants contained in the supplied membrane. This precaution applies equally to any device where this particular surfactant/barrier combination is used and where liquid moves a relatively long distance to a switchable barrier and remains there for some time prior to switching.

Subsequent application of liquid and fluid flow is as described with reference to Figure 2b, except it was found that the provision of the additional impervious line 21a straightened the liquid front as it approached the surfactant line 27 and so promoted more even switching of the barrier 26.

The effectiveness of the coumarone resin barrier and ODG surfactant combination was separately evaluated with water, urine, plasma and serum.

EXAMPLE 3

Referring to Figure 3, a polyester backed 8 μm nitrocellulose membrane 30 is divided into channels by impervious pen lines 31 as described for Example 2a. A vertical first channel 32 leads to left and right inner and

outer channels 33l,33r;34l,34r at its upper end, its lower end defining the base of the device. Each outer channel 34l,34r extends around the periphery of the membrane 30 and has an enlarged end region 35l,35r remote from the vertical first channel 31. The right outer channel 34r has a convoluted region 36 so that the length of the right outer channel 34r is greater than that of the left outer channel 34l. The left and right inner channels 33l,33r are short in relation to the outer channels 34l,34r and lead into the leftmost and rightmost ends respectively of a horizontal reaction channel 37. The right inner channel 33r also leads into a first overflow channel 38 which is otherwise closed. The reaction channel 37 is provided with a capture line 39 (containing immobilised antibody to analyte of interest) at its midpoint and a gold conjugate containing region 40 spaced to the left of the capture line 39. In other embodiments, the gold conjugate is replaced by different visualising agents.

The enlarged end regions 35l,35r of the left and right outer channels 34l,34r are separated from second and third overflow regions 41l,41r respectively by a first "Type 1" barrier 42l,42r (prepared by centrifuging the silver paint contained in pen type 8700 at 750 g for 20 minutes, diluting the supernatant therefrom with 5% white spirit and plotting with a 0.5 mm pen at 1 cm/s) and from the left and rightmost ends respectively of the reaction channel 37 by a second similar barrier 43l,43r perpendicular to the first barrier 42l,42r. The first and second barriers 42l,42r;43l,43r were dried at 22°C for 2 minutes. Each enlarged end region 35l,35r contains a line of surfactant 44l,44r (2.5% ODG plotted using a 0.7 pen at 5 cm/s, and dried at 35° C for 20 minutes) spaced from and parallel to the respective first and second barriers 42l,43l;42r,43r.

In use, sample being analysed for an analyte of interest is applied to the gold conjugate containing region 40 and the vertical channel 32 of the device is placed into water (or other liquid) which wicks up the vertical channel 32 and into the left and right inner and outer channels 33l,33r,34l,34r. From the left and right inner channels 33l,33r, water flows into the left and right end respectively of the reaction channel 37. Flow from the left end passes through the gold conjugate containing region 40 and washes gold-analyte complex (and gold-conjugate and unreacted sample) towards the capture line 39. Since part of the flow from the right inner channel 33r is diverted into the first overflow channel 38, the flow into the right end of the reaction channel 37 is less than into the left end. Thus, there is net flow in the reaction channel 37 from left to right and the sample is carried across the capture line 39 (left to right) for a **first time**, where it reacts with the immobilised antibodies. When the first overflow channel 37 is filled, flow in channel 38 substantially stops.

Simultaneously, water continues to flow along the relatively longer outer channels 34l,34r, but because the left outer channel 34l is shorter than the right outer channel 34r, water arrives at the enlarged end region 35l of the left outer channel 34l before the enlarged end region 35r of the right outer channel 34r. A part-circular water front flows into the surfactant line 44l and water now containing surfactant flows simultaneously to the first and second barriers 42l,43l. The surfactant switches the barriers 42l,43l so that liquid can flow therethrough. Thus, the enlarged end region 35l of the left outer channel 34l is in communication with both the left end of the reaction channel 37 and the second overflow channel 41l. Since the

second overflow channel 41l is dry, liquid is drawn into the second overflow channel 41l from the enlarged end region 35l of the left outer channel 34l and the left end of the reaction channel 37. This causes right to left flow in the reaction channel 37 and the sample/gold conjugate passes through the capture line 39 (right to left) for a **second time**.

Meanwhile, water continues to flow in the right outer channel 34r and into its enlarged end region 35r. A part-circular water front flows into the surfactant line 44r and water now containing surfactant flows simultaneously to the first and second barriers 42r,43r. The surfactant switches the barriers 42r,43r so that liquid can flow therethrough. Thus, the enlarged end region 35r of the right outer channel 34r is in communication with both the right end of the reaction channel 37 and the third overflow channel 41r. Since the third overflow channel 41r is dry, liquid is drawn into the third overflow channel 41r from the enlarged end region 35r of the right outer channel 34r and the right end of the reaction channel 37. This causes left to right flow in the reaction channel 37 and the sample/gold conjugate passes through the capture line 39 (left to right) for a **third time**. This last movement along the reaction channel 37 moves the gold clear of the capture line 39 and provides a wash step so that the capture line 39 can easily be seen.

It will be understood that the distances moved by the sample/gold across the capture line 39 is determined by the volume of the first, second and third overflow regions 38,41l,41r. Such a device offers improved sensitivity when using a small sample volume by allowing sample and reagents to pass several times over the capture line 39.

EXAMPLE 4

Referring to Figure 4, a polyester backed 8 µm nitrocellulose membrane 50 is divided into channels by impervious pen lines of neat Lumocolor ink as described for Example 2a. The membrane 50 has a substantially circular portion 50a and a rectangular base 50b. The periphery of the membrane 50 is marked with an impervious line 51 except for a bottom edge 52 of the base 50b which serves as a liquid inlet. An impervious line 53 extends generally towards the centre of the circular portion 50a from each side of the base 50b where the base 50b intersects the circular portion 50a to define an open-ended tapering first channel 54. A switchable barrier 55 extends partly across the first channel 54 at the same radius as the impervious line 51 marking the periphery of the membrane 50. A further impervious line 56 extends generally towards the centre of the circular portion 50a from each end of the switchable barrier 55 to define an open-ended tapering second channel 57 wholly within the first channel 54. Within the second channel 57 is disposed a gold conjugate (or other visualising agent) containing region 58 spaced above a line of surfactant 59 (1.25% ODG plotted using a 0.5 pen at 5 cm/s, and dried at 35° C for 10 minutes). A capture line of immobilised antibody 60 is positioned above the first channel 54 and is parallel to and above the switchable barrier 55.

In use, the base 50b of the membrane 50 is placed in a liquid sample. Liquid wicks into the first channel 54 and around the second channel 57, upward flow into the second channel 57 being prevented by the switchable barrier 55. Liquid then flows down into the second channel 57

(moving the gold conjugate and surfactant line towards the switchable barrier 55) and upwards out of the first channel 54 where it forms a part-circular front. The front passes through the capture line 60 and outwards towards the periphery.

The downwardly flowing sample together with the gold conjugate in the second channel 57 wicks into the surfactant line 59 and continues until it contacts the switchable barrier 55. The surfactant acts to convert the capillaries of the barrier 55 from non-wetting to wetting in order for liquid to flow therethrough. However, flow through the barrier 55 does not occur immediately.

Since the switchable barrier 55 is located at about the same radius as the periphery of the membrane 50, outward flow reaches the periphery at about the same time as downward flow in the second channel 57 reaches the switchable barrier 55. When the saturation at the switchable barrier 55 has reached a critical value (dependent upon the barrier/surfactant combination) the barrier 55 becomes permeable. Outward flow towards the periphery continues drawing liquid through the second (and first) channels 57,54, thereby causing gold conjugate to flow over the capture line 60 until total saturation occurs. Any analyte present in the sample will be visualised at the capture site 60, with any unbound material being washed clear.

Switching of different barriers was investigated using water as the sample and the results were as follows:-

1 A "Type 2" barrier (prepared by diluting 1ml of type 1 barrier material with 250 µl of white spirit and 250 µl of ethanol and plotted using a 0.5 mm pen at 5 cm/s) opened at 3 minutes 50 seconds.

2 White paint barrier plotted using a 0.7 mm pen at 1 cm/s opened at 1 minute 10 seconds (before liquid reached the periphery).

3 1 part Lumocolor in 4 parts 1-propanol barrier plotted using a 0.5 mm pen at 5 cm/s opened at 3 minutes 30 seconds.

This embodiment provides a means of first delivering a large volume of sample through an antibody capture line followed by a visualising agent.

EXAMPLE 5

Referring to Figure 5, a rectangular polyester backed 8 µm nitrocellulose membrane strip 61 is marked around its periphery by an impervious pen line 62 of neat Lumocolor ink as described for Example 2a except for a short bottom edge 63 which serves as a liquid inlet. Parallel to the bottom edge 63, a first Type 2 material switchable barrier 64 (plotted using a 0.5mm pen at 5 cm/s) extends partly across the strip 61. An impervious line 65 extends perpendicularly from each end of the first barrier 64 away from the bottom edge 63 to define a channel 66 having a closed lower end and an open upper end. Within the channel 66 is a colorimetric reagent containing region 67 spaced above a line of surfactant 68 (2.5% ODG plotted using a 0.7mm pen at 5 cm/s, and dried at 35° C for 10 minutes). Above the channel 66, a capture region 69 is defined within a rectangular line of second Type 2 switchable barrier 70. The capture region 69 contains immobilised antibody and an enzyme conjugate. In this embodiment, the colorimetric reagent is one which interacts with the

enzyme conjugate and not the analyte itself. A rectangular line of surfactant 71 (also 2.5% ODG) surrounds the capture region 69 and is spaced from the second barrier 71.

In use, about 3 µl of sample to be analysed for the analyte of interest is applied onto the capture region 69 eg. By pipetting. The sample is contained within the capture region 69 by the second switchable barrier 70. The enzyme conjugate is solubilised and if the analyte of interest is present in the sample it becomes bound to the immobilised antibody and the enzyme conjugate. The bottom edge 63 of the membrane is then placed in water (or other suitable diluent). Water flows along the strip 61 around the channel 66 but cannot flow into the channel 66 from below because of the first barrier 64. Flow continues along the strip 61 towards the rectangular line of surfactant 71. When surfactant reaches the second barrier 70, the second barrier 70 is switched to allow water to flow therethrough. The capture region 69 is than totally open to flow and sample and any excess enzyme conjugate is washed upwards away from the capture region 69.

During this time flow enters the channel 66 from above and the colorimetric reagent and line of surfactant 68 move towards the first barrier 64. When the barrier 64 is switched, flow in the channel 66 is reversed and the colorimetric reagent moves over the capture region 69. If the analyte of interest is present, the colorimetric substrate reacts with the enzyme conjugate to produce visible colour at the capture region 69. In this embodiment, the relative dimensions of the device and positioning of the various elements of the device are such that the device becomes totally

saturated at substantially the same time as the colorimetric reagent reaches the capture region, so that the colorimetric reagent remains at the capture region.

The device is also designed so that the first barrier 64 is switched after the second barrier 70 to ensure that the colorimetric reagent passes into the capture region 69 and not around it.

This embodiment provide a means of analysing sample using a multi-reagent assay. It will be noted that excess conjugate is automatically washed away from the capture region 69 prior to arrival of colorimetric reagent at the capture region 69. The device is particularly suited for screening assays and offers an automatic non-instrumental alternative to microtitration plate assays.

Example 5 describes a device having a single capture region 69, but in a modification, several capture regions are provided so that a number of samples can be analysed in a single assay.

EXAMPLE 6

Referring to Figure 6a, a rectangular 12 μm nominal pore size unsupported nitrocellulose membrane strip 80 (type AE 100, Schleicher & Schuell, Dassel, Germany) is marked with impervious pen lines 81 of neat Lumocolor ink parallel to and marginally spaced from its long side edges 82. Parallel to a first short side edge 83, a first Type 2 switchable barrier 84 extends partly across the strip 80 (plotted using a 0.7 mm pen at 1 cm/s and dried at 22° C for 2 minutes). An impervious line 85 extends

perpendicularly from each end of the first barrier 84 away from the first side edge 83 towards a second short side edge 86 to define a channel 87 having a closed first end and an open second end. Within the channel 87 is a gold conjugate containing region 88 spaced to the left of a line of surfactant 89 (10% ODG plotted using a 0.7 pen at 5 cm/s). Disposed between the second short side edge 86 and the channel 87, a capture region 90 is defined within an elliptical ring of a second Type 2 switchable barrier 91. The capture region 90 contains immobilised antibody. An elliptical ring of surfactant 92 (also 10% ODG) surrounds the capture region 90 and is spaced from the second barrier 91.

Referring to Figure 6b, the unsupported membrane strip 80 of Figure 6a is adhered to a polyester backing 93 (0.025 mm thick type 7759 film coated on one side with acrylic adhesive AS-110, Adhesives Research Ireland Ltd, Limerick, Ireland) and mounted to a housing 94. It should be noted that for unsupported membrane material, one face tends to have larger pores than the other face. Under low liquid pressure loads, liquid does not flow easily through the membrane from the large pore face to the small pore face. The polyester backing 93 is adhered to the large pore face of the membrane strip 80 (identified by being less shiny than the small pore face). The backing 93 is provided with an aperture 95 therethrough which is aligned with the capture region 90 of the membrane strip 80.

The housing 94 is machined from Perspex acrylic sheet (ICI, Darwen, UK) and is generally cuboidal. First and second troughs 96,97 extend partly across the width of the housing 94. The second trough 97 is deeper than the first trough 96 and is in communication therewith by virtue of a flow

passage 98. The base of the flow passage 98 is higher than the base of the first trough 96 so that a lip 99 is defined between the first and second troughs 96,97. Within the first trough 96 is embedded a peg 100 which projects vertically. The polyester backed membrane is mounted flat on an upper surface of the housing 94 in such a position that the capture region 90 is directly above the peg 100 in the first trough 96. An end portion of the membrane is bent downwardly so that its first short side edge 83 is in contact with the base of the second trough 97.

In use, sample is applied to the capture region 90 using a pipette (in an alternative embodiment, not shown, a funnel is positioned above the capture region 90 and loaded with a pre-determined volume of sample which is automatically applied to the membrane at a desired rate) and is constrained from flowing into the surrounding membrane by the second barrier 91. Sample quickly flows vertically through the thickness of the membrane 80 from the small pore face of the membrane 80 to the large pore face and through the aperture 95 in the polyester film 93. If the analyte of interest is present it will become bound to the immobilised antibody capture region 90. Pin 100 helps to guide flow down into the first trough 96. When sufficient sample is applied, sample flows over the lip 99 into the second trough 97 and wets the first short side edge 83 of the membrane 80. Sample flow is then as described with reference to Example 5 resulting in (i): washing of unbound sample from the capture region 90, (ii) flow of gold conjugate over the capture region 90 and (iii) flow of excess gold conjugate away from the capture region 90.

This embodiment allows a large volume of sample to be analysed, important if the analyte of interest is present in low concentrations. Furthermore, the sample volume, after passing vertically down through a relatively small capture site is directed to the end of the strip and flows back through the capture site horizontally. It is particularly suited for detecting bacteria (eg. Coliforme) in diluted samples as is often required in environmental tests.

In a modification of the above embodiment, the troughs 96,97 contain porous or absorbent material to absorb excess liquid and prevent it from escaping from the housing 94 after use. It will be understood that the purpose of the troughs 96,97 in the housing 94 is to enable a predetermined volume of sample to be applied to the capture region 90 before flow is initiated along the membrane 80. The same effect may be achieved by other methods. For example, in another modification, sample flowing through the membrane passes into porous material, or a capillary channel which is in abutment with the first short edge of the membrane (see Example 8). Suitable porous materials include glass fibre fleece, cellulose fibre fleece, polymeric sponge-like material and poly(isobutylene-co-maleic acid) sodium salt, with good absorbent properties.

EXAMPLE 7

Referring to Figure 7a, a rectangular 8 µm nominal pore size unsupported nitrocellulose membrane strip 100 (type AE99, Schleicher and Schuell) with the small pores uppermost, is marked with an impervious pattern of pen lines using neat Lumocolor ink (plotted using a 0.7 mm pen at 3 cm/s

and dried at 22°C for 2 minutes) to define an inner channel 102 between a pair of outer channels 104, all of which channels 102,104 extend from a first (lower) common region 106 to a second (upper) common region 108 (references to "upper" and "lower" are in relation to the orientation depicted in Figure 7a). Specifically, the inner channel 102 is defined by a pair of spaced apart parallel impervious lines 110 which are joined at their lower ends by a first line of coumarone resin 112 (prepared as described in Table 10) serving as a switchable barrier (plotted using a 0.7 mm pen at 3 cm/s and dried at 22°C for 2 minutes). An additional impervious line 114 extends perpendicularly to and is spaced above the first pair of parallel impervious lines, with the ends 114a of the additional impervious line 114 extending a short distance vertically downwardly. The portion of the additional impervious line 114 extending perpendicularly to the parallel impervious lines 110 being longer than the width of the inner channel 102. The ends 114a of the additional impervious line 114 and the upper ends of the parallel impervious lines 110 are connected by a respective one of a pair of additional coumarone resin (switchable barrier) lines 116. It will therefore be understood that in the configuration described, the inner channel 102 is completely enclosed.

The outer channels 104 are defined by further impervious lines 118, as well as the parallel impervious lines 110 and the additional coumarone resin lines 116 described above. The lower ends of the outer channels 104 are mutually parallel, with the upper ends mutually diverging towards the edges of the membrane strip 100.

A capture region 120 containing immobilised antibody is provided towards the upper end of the inner channel 102 (between the parallel impervious lines 110), with a gold conjugate region 122 being provided below the capture region 120 also in the inner channel 102. A line of surfactant 124 (10% solution of ODG plotted using a 0.7 mm pen at 5 cm/s) extends downwardly along one of the outer channels 104, underneath the inner channel 102 and upwardly along the other outer channel 104. In the embodiment shown, a further coumarone switchable barrier 126 is provided immediately below the first coumarone resin line 112 and a further line of 10% ODG surfactant 128 is provided below the first line of 10% ODG surfactant underneath the inner channel 102. These further lines of coumarone resin barrier 126 and ODG surfactant 128 are optional, but have been found to promote more reliable switching of the coumarone resin barrier 112 in use.

Referring to Figure 7b, adhered beneath (to the right in Figure 7b) the nitrocellulose membrane strip 100 is a polyester backing 130 (0.254 mm thick type 8565 film coated on one side with acrylic adhesive AS-110, Adhesives Research). The backing 130 is provided with an aperture 132 (the edge of the aperture 132 being coated with 10% ODG solution applied from the direction of the non-adhesive side) and is shorter in length than the nitrocellulose membrane strip 100. The backing 130 is adhered to the membrane strip 100 such that the aperture 132 is directly beneath the capture region 120 containing the immobilised antibody and so that the first (lower) common region 106 of the membrane strip 100 extends below the backing 130 (Figure 7c). A strip of silk 134 (or other think fabric such as synthetic fabric type PE33HC (ZBF, Zurich) is adhered

across the backing 130 so as to cover the aperture 132. This is achieved by masking the region of the aperture 132 and applying a light spray of adhesive (Spraymount adhesive, 3M United Kingdom PLC., UK) to the surface of the backing 130 (Figure 7d).

It should be noted that if the synthetic fabric is used, this is first impregnated with 10% ODG solution and dried before being used. A strip of porous material 136 , in this case Vileda Sunsplash [TM] (Freudenberg Household Products LP, Rochdale) is adhered to the backing 130 (above and below the silk strip 134, to the silk strip 134 itself and the lower end of the nitrocellulose membrane strip 100 – see Figure 7e). This is achieved by applying a further light spray of Spraymount adhesive to the fabric side of the device and gently compressing the Vileda Sunsplash porous material 136 onto the device. It should be noted that the porous material is only in direct contact with the membrane at the lower end of the latter. The strip of porous material 136 is narrower than the membrane strip 100 and does not extend as far as the side edges of the latter (Figure 7e).

In use, the device is supported with the membrane strip 100 in a horizontal position and sample applied to the capture region 120 using a pipette. Although it will be appreciated that in alternative embodiments, a sample application well may be incorporated to a housing for the device and positioned over the capture region 120 (not shown). Sample is constrained from flowing out of the inner channel 102 by the impervious lines 110, 114 and the coumarone resin lines 112,116. Thus, as more sample is applied, it flows vertically through the thickness of the

membrane strip 100 and through the aperture 132 in the polyester backing 130. If the analyte of interest is present, it will become bound in the immobilised antibody capture region 120. Sample flowing through the aperture 132 in the polyester backing 130 flows into the silk 134 and then into the porous material 136. Sample continues to flow along the porous material 136 and, because it is in direct contact with the membrane strip 100, back into the membrane strip 100 into the first (lower) common region 106 of the latter. The capillarity of the porous material 136 is higher than that of the aperture 132, and the volume of the porous material 136 is chosen such that after the entire sample has been applied, any free liquid in the inner channel 102 is absorbed into the porous material 136 and the aperture 132 is clear of sample. As the sample flows from the first (lower) common region 106 towards the second (upper) common region 108, the ODG surfactant lines 124,128 are moved towards the coumarone resin barriers 112,126 at the lower end of the inner channel 102 which are consequently opened by the action of the ODG surfactant. Sample continues along the outer channels 104, but does not flow through the inner channel 102 since the additional coumarone resin barriers 116 have not yet opened (flow of sample through a channel requires an outlet in that channel). When the ODG initially present in the outer channels 104 is moved by sample flow into the additional coumarone resin barriers 116, the latter are switched thereby providing an outlet from the inner channel 102. As a result, sample flows along the inner channel 102 so as to wash the gold conjugate over the analyte capture region 120 where it becomes bound if the analyte of interest is present. Further flow causes unbound gold

conjugate to clear the capture region 120 in order for the result to be clearly observed.

It will be understood that sample cannot flow from the porous material 136 upwardly through the aperture 132 thereby causing flow distortion in the inner channel 102, because the aperture 132 is already clear of sample. Furthermore, the silk 134 prevents fibres projecting from the surface of the porous material 136 from forming a fluid pathway back through the aperture onto the membrane strip 100. Thus, in alternative embodiments (not shown) the Vileda Sunsplash material is replaced by an absorbent material without projecting surface fibres thereby removing the necessity for the silk 134 between the polyester backing 130 and the porous material 136.

As with Example 6, this embodiment allows a large volume of sample to be analysed, with most of the sample flowing through the capture region first vertically downwards and subsequently horizontally through the capture region.

EXAMPLE 8

Referring to figure 8, a semi-quantitative assay device comprises a generally rectangular (56 mm x 46 mm) strip 140 of polyester backed 8 µm nitrocellulose membrane. A detection solution application region 142 is defined by permanent impervious lines 144a,b,c formed by the application of neat Lumocolor ink (each impervious line 144a,b,c is formed from three 0.5 mm spaced parallel lines plotted using a 0.5 mm pen at 4 cm/s and dried at 22°C for 2 minutes, each of the parallel lines

merging to form a thickened line) and a switchable barrier 146 of coumarone resin (prepared according to table 10 and plotted using a 0.5 millimetre pen at 5 cm/s). The permanent impervious lines form first 144a and second 144b parallel sides and base 144c of the detection solution application region 142 with the coumarone resin line 146 defining the top of the detection solution application region 142 (as viewed in Figure 8). A first additional permanent impervious lines 148a defines a sample application region 150 positioned to one side of the detection solution application region 142 and with a second additional impervious line 148b defines a fluid flow channel 152 located above the detection solution application region 142. The sample application region 150 is in communication with the fluid flow channel 152 via a first outlet 154. In a direction moving upwardly from the detection solution application region 142, the fluid flow channel 152 has a lower region 152a of constant width and a tapering region 152b to a relatively narrow second outlet 156 leading to a waste region 160. The first side 144a of the detection solution application region 142, the first outlet 154 and an upper part of the impervious line 148a defining the first side of the fluid flow channel 152 are collinear. The tapering of the fluid flow channel 152 is achieved by the impervious line 148b defining the second side of the fluid flow channel 152 (remote from the sample application region 150 and first outlet 154) which converges towards the first side of the fluid flow channel 152 as it extends towards the second outlet 156. A line of capture antibody 158 extends parallel to and above the coumarone resin line 146 from directly above the first outlet 154 to the second side of the fluid flow channel 152.

In use, sample is pipetted into the sample application region 150 and flows through the first outlet 154 into the lower region 152a of the fluid flow channel 152. Initially, the wet/dry sample front flowing from the first outlet 154 is in the form of a quadrant of a circle. However, the flow pattern changes as the wet/dry front approaches and passes through the second outlet 156. The geometry of the fluid flow channel 152 is such that at the first side (adjacent the sample application region 150 and first outlet 154) , flow is substantially upwards whereas towards the second side of the fluid flow channel 152 (remote from the first outlet 154) flow becomes increasingly stagnated. As a result, more sample passes through the line of capture antibody 158 towards its end directly above the first outlet 154 than at its end remote from the first outlet 154. Once the sample is absorbed into the membrane strip 140, detection solution containing surfactant is applied to the detection solution application region 142, the surfactant causing the coumarone resin switchable barrier 146 to open. The detection solution, then moves over the antibody capture line 158 with a substantially linear flow front. It will be understood that particular assay characteristics such as dynamic range, sensitivity, and degree of linearity can be provided by a combination of device geometry and the formulation of the agents used.

As an example of the use of this device as a semi-quantitative determination of analyte concentration, the following solutions were used:-

(i) Capture antibody: 2 mg/ml of rabbit polyclonal myoglobin antibodies (Spectral Diagnostics, Toronto).

(ii) Detection solution: 40 nm gold sol (British Biocell International (BBI), Cardiff) coated with 1.5 µg/ml of a mouse monoclonal myoglobin antibody (Spectral Diagnostics) at an optical density of 1 according to BBI instructions. The final detection solution contains 6% bovine serum albumin, 2.5% ODG, gold conjugate at an optical density of 0.5 and phosphate buffered saline (PBS) at pH 7.4.

(iii) Sample solutions: 6% bovine serum albumin in PBS at pH 7.4 spiked with the indicated amounts of rec.myoglobin (Spectral Diagnostics).

For each run, 60 µl of sample solution was applied to the sample application region and then 100 µl of detection solution was applied to the detection solution application region. The results shown in table 13 below indicate a strong correlation between visualised gold-analyte complex and initial analyte concentration.

Table 13: Correlation of visualised signal length to analyte concentration

Myoglobin (ng/ml)	Signal length (mm)
0	0
40	3
200	7
400	12
2000	18
capture line length: 20 mm total test time: 10 minutes	

In alternative embodiments, one or both of the sample application region and detection solution application region can be provided with a sample

application well and detection solution well respectively incorporated into a housing for the device. Alternatively, the device may be supplied with the gold conjugate solution (preferably with a reagent to prevent unspecific binding to the membrane) already dried onto the detection solution application region. Additionally, ODG may be dried onto the detection solution application region 142. In these cases, only diluent need be applied to bring the gold into solution when required. In yet another embodiment, the line of capture antibody is replaced by a number of dots, the range of analyte concentration being determined by the number of visualised dots rather than the length of a visualised line.

CLAIMS

1. A fluid-flow control device comprising:-

(i) a fluid flow path,

(ii) a fluid flow barrier at a location in the flow path, and

(iii) barrier release means in the flow path which, in use, is moveable by fluid flowing in the flow path into contact with the flow barrier so as to permit fluid flow through said location.

2. A device as claimed in claim 1, wherein said fluid is an aqueous liquid.

3. A device as claimed in claim 1 or 2, wherein the fluid flow barrier is a non-wetting region in the flow path and the release means is a wetting agent capable of wetting said non-wetting region, so as to enable flow of liquid through said region.

4. A device as claimed in any preceding claim, wherein the fluid flow barrier is a hydrophobic material and the release means is a surfactant.

5. A device as claimed in claim 4, wherein the fluid flow barrier is selected from the group consisting of waxes, resins, synthetic polymers, inks and paints.

6. A device as claimed in claim 4 or 5, wherein the release means is selected from the group consisting of octyl-β-D-glucopyranoside, dioctyl

sulfosuccinate sodium salt, polyoxyethylene (23) dodecyl ether and polyoxyethylene (20) sorbitan monolaurate.

7. A device as claimed in any preceding claim wherein, the flow path is defined by interstices of a porous medium.

8. A device as claimed in claim 7, wherein the fluid flow barrier is impregnated into and immobilised on the porous medium.

9. A device as claimed in claim 7 or 8, wherein the barrier release means is impregnated into the porous medium and is soluble in the flowing fluid.

10. A device as claimed in any preceding claim, wherein the device is constructed so that, in use, there is at least one region in the flow path where the direction of fluid flow is different, after fluid flow through said location is initiated compared with the direction of fluid flow in that region before fluid flow through said location is initiated.

11. An assay device for an analyte comprising:-

(i) a flow path for a liquid defined by interstices in a porous medium,

(ii) at least one liquid flow barrier on the porous medium at a location in the flow path,

(iii) at least one barrier release means which is impregnated into the porous medium, which is soluble in the liquid, and which, in use, is moveable by liquid flowing in the flow path into contact with the flow barrier so as to permit liquid flow through said location, and

(iv) an analyte capture region in the flow path.

12. A device as claimed in claim 11, wherein the device is provided with a visualising agent application zone adapted to receive a visualising agent.

13. A device as claimed in claim 11, wherein an analyte visualising agent is provided at a zone in the flow path, said visualising agent being capable of interacting with the analyte to indicate the presence of the analyte.

14. A device as claimed in claim 13, wherein the visualising agent is immobilised in the capture region and also serves to immobilise the analyte in the capture region.

15. A device as claimed in claim 13, wherein the analyte capture region includes an immobilised analyte binding substance which serves to immobilise analyte in the capture region and the visualising agent is upstream of the capture region so that in use, it flows through the capture region.

16. A device as claimed in any one of claims 11 to 15, wherein a sample application zone is provided.

17. A device as claimed in any one of claims 11 to 16, wherein a flow path geometry is selected which causes, in use, a predetermined difference in fluid flow within the analyte capture region so that distribution of

analyte within the analyte capture region is non-uniform in a predictable manner, whereby the amount of analyte can be determined in at least a semi-quantitative manner.

18. A device as claimed in claim 16, wherein the sample application zone corresponds to the analyte capture region or the visualising agent application zone of claim 12.

19. A device as claimed in claim 16 or 18, wherein the sample application zone is surrounded by an additional flow barrier and an associated barrier release means.

20. A device as claimed in any one of claims 11 to 18, wherein the sample to be analysed is included in the liquid which flows, in use, along the flow path.

21. A device as claimed in any one of claims 11 to 20, wherein at least two barriers and at least two barrier release means are provided and arranged so that, in use, some liquid flows over the analyte capture region at least twice.

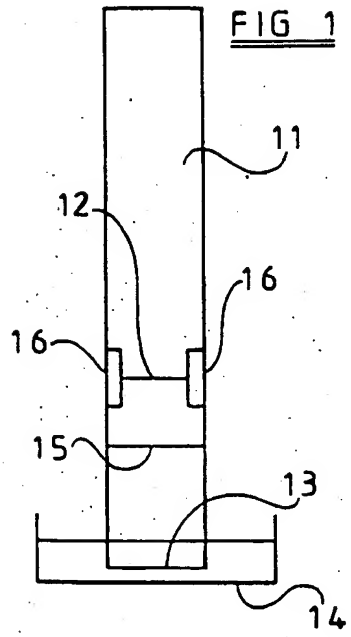


FIG 1

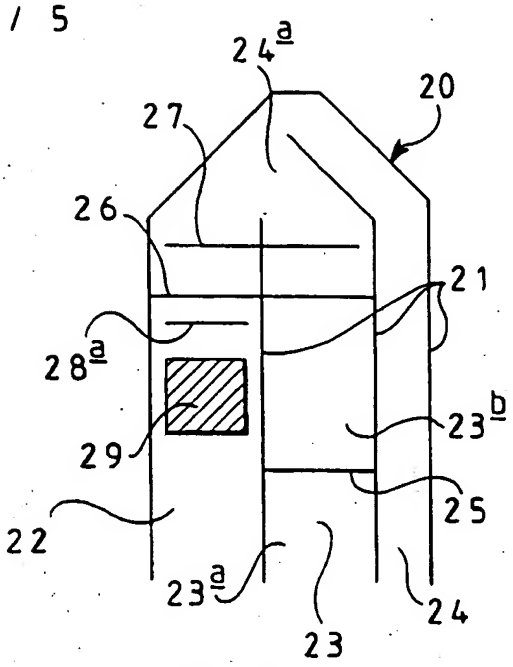


FIG 2a

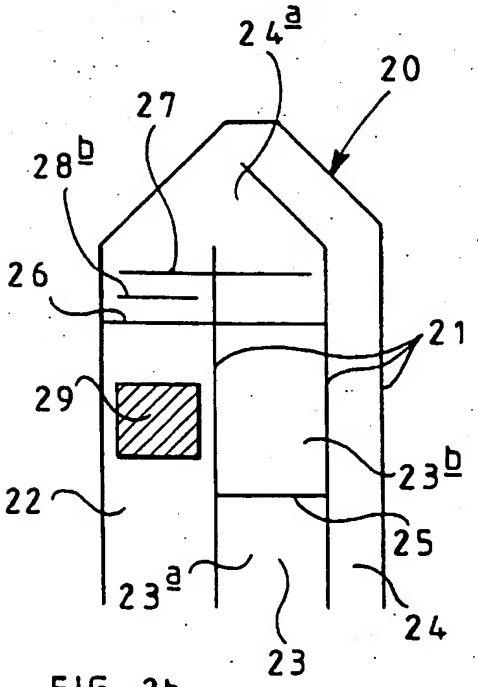


FIG 2b

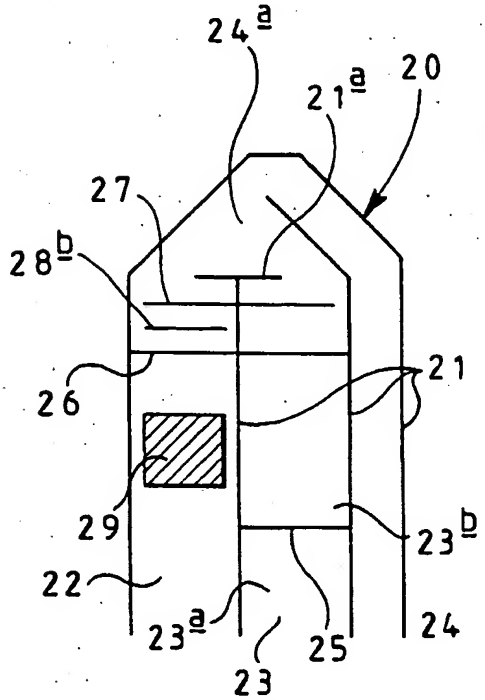
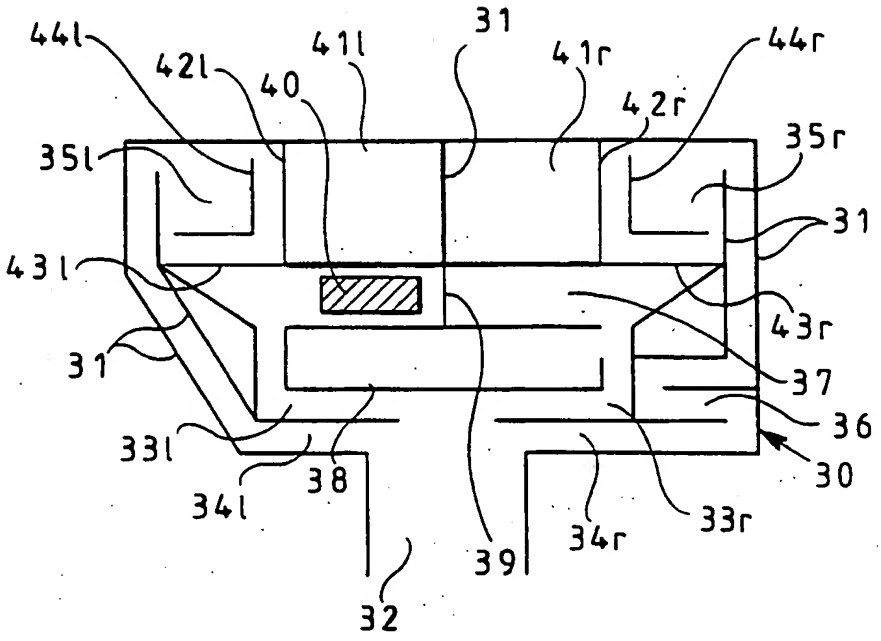


FIG 2c

FIG 3



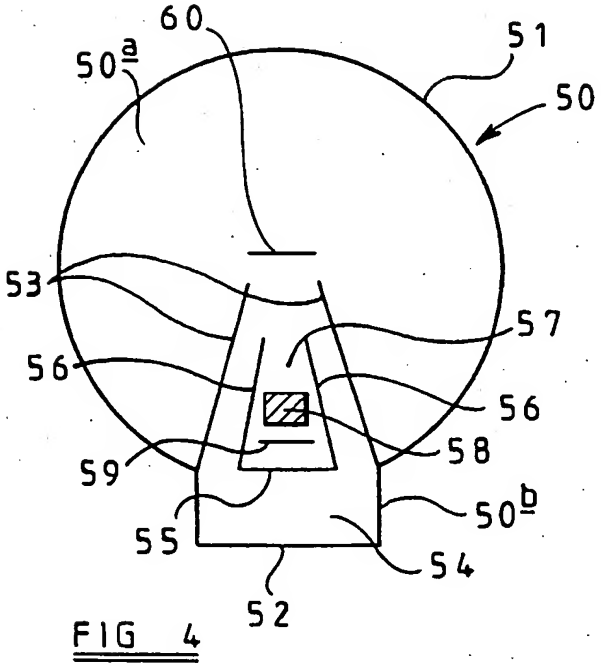


FIG 4

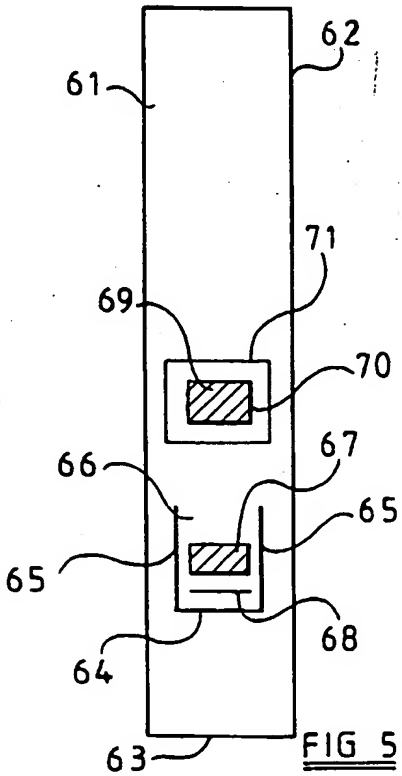
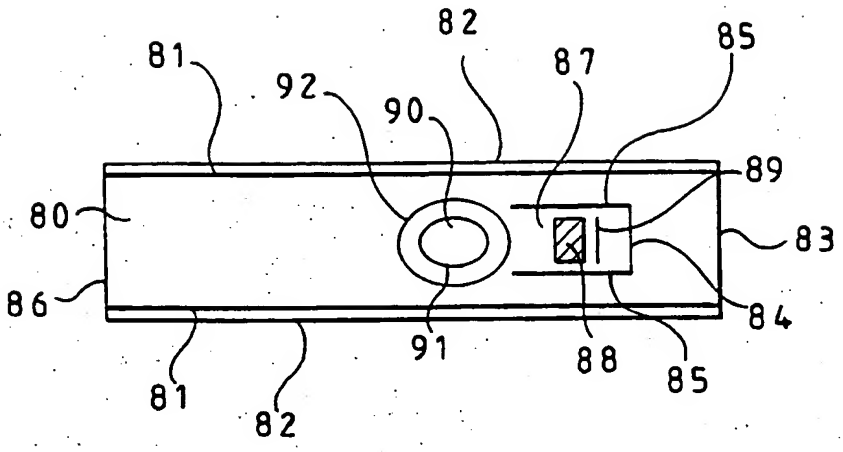


FIG 5

FIG 6a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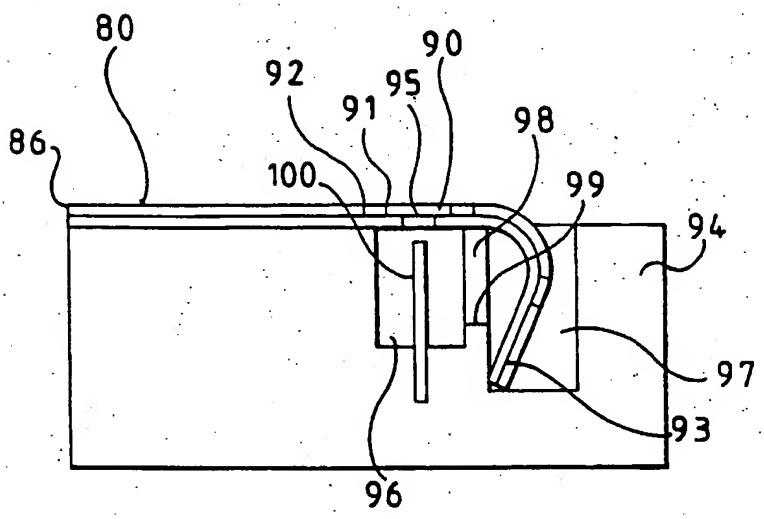
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FIG 6b

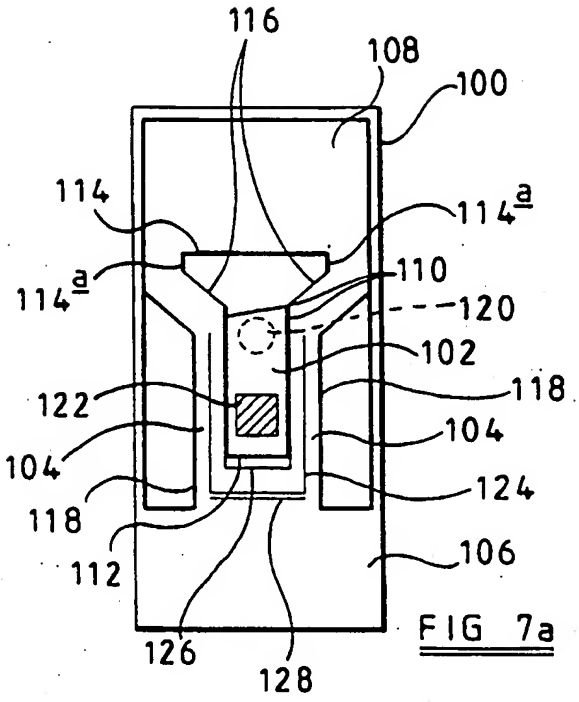

FIG 7a

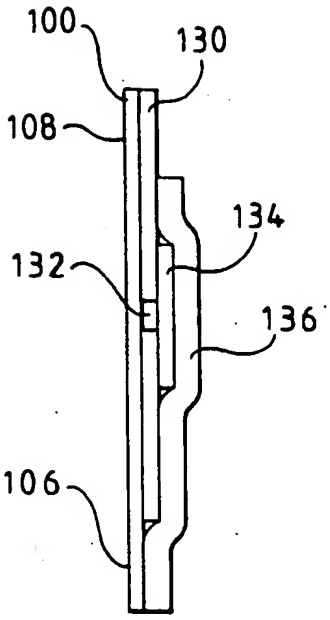

FIG 7b

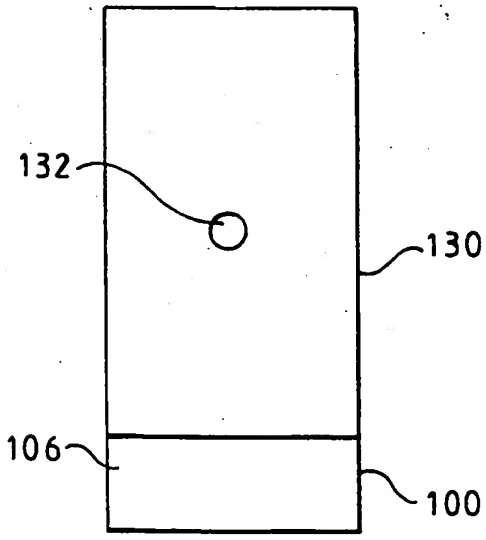

FIG 7c

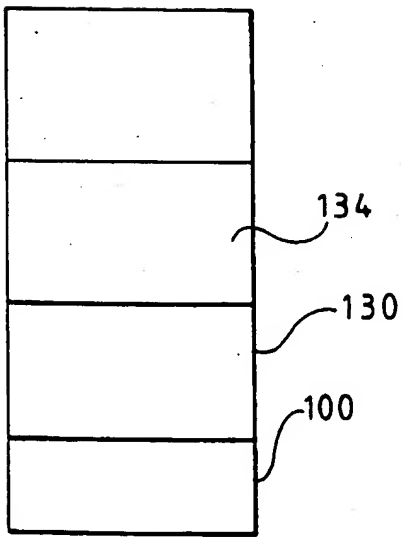

FIG 7d

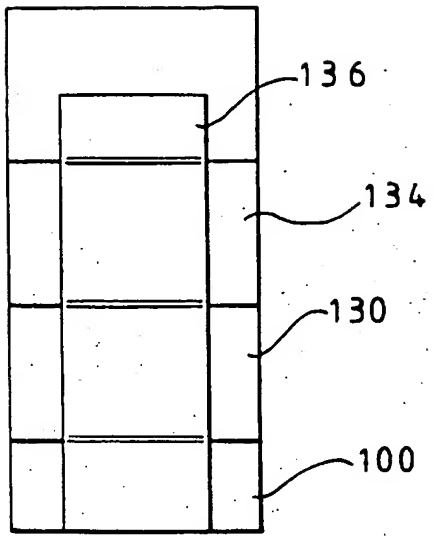
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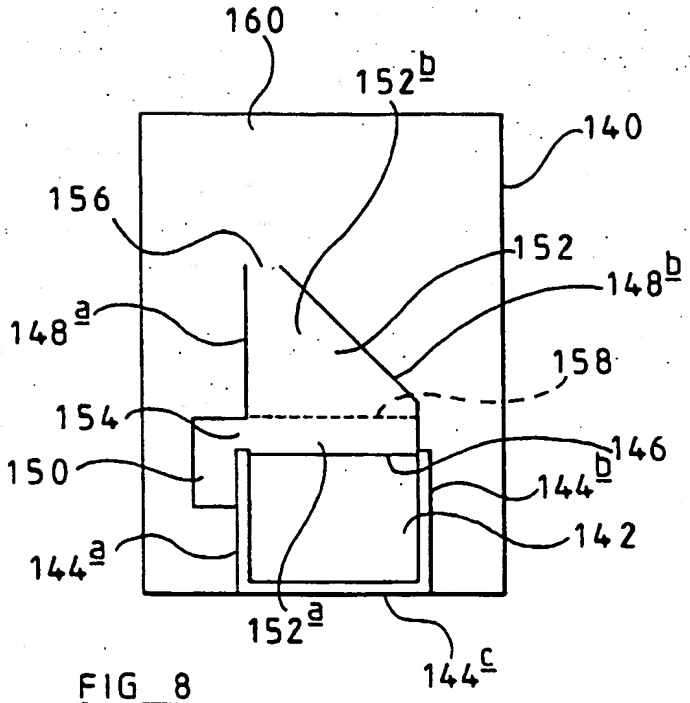
FIG 7e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144a
152a
144c

FIG 8

INTERNATIONAL SEARCH REPORT

In...national Application No
PCT/GB 00/03825

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/558 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 96 24060 A (BRITISH BIOCELL INT ;CHANDLER JOHN ANTHONY (GB)) 8 August 1996 (1996-08-08) page 8, line 14 - line 24 page 27, line 29 -page 29, line 10; figures 10,13 figure 7; example 5 ---	1-21
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2001	12/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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International Application No
PCT/GB 00/03825

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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